*EMBRYONIC CELL ADHESIVENESS: DO SPECIES DIFFERENCES EXIST AMONG WARM-BLOODED VERTEBRATES?**

By Morton L. Burdick and Malcolm S. Steinberg

DEPARTMENT OF BIOLOGICAL SCIENCE, FLORIDA STATE UNIVERSITY, TALLAHASSEE; AND DEPARTMENT OF BIOLOGY, PRINCETON UNIVERSITY

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Abstract.—Mouse and chick embryonic myocardial cells sort out from each other in aggregates of heart ventricle cells. This result invalidates the generalization that embryonic cells of a given tissue from different warm-blooded vertebrate species possess identical cell-sorting properties.

Aggregation and tissue reconstruction by dissociated embryonic cells have been studied to learn the mechanisms controlling the movements and arrangements of cells in embryos.[1–6] In aggregates containing initially intermixed cells derived from two or more different tissues, the cells typically segregate according to tissue type. This process is widely regarded as a form of cellular "self-recognition."

One approach toward greater understanding of this morphogenetic recognition system is to compare its operation among different animal species. Moscona and his associates have prepared aggregates containing mouse and chick embryonic cells derived from the same type of tissue. They reported that, for limb precartilage,[7, 8] liver,[7, 8] skin,[9] neural retina,[10] and embryonic kidney,[11] the mouse and chick cells of the same type did not sort out from each other according to species. These results have suggested the generalization that, for any one embryonic cell type, the properties responsible for cell sorting are indistinguishable among even very distantly related warm-blooded vertebrate species.

Recently, we have observed that mouse and chick embryonic heart myocardial cells do not conform to this generalization. We have found that these cells sort out well from each other, and we wish to report these results.

Materials and Methods.—Litters of mouse embryos (CD-1 strain, random-bred, Charles River Mouse Farms, Wilmington, Mass.) were staged according to the structure of their limbs, using the criteria of Gruneberg.[12] Mouse heart ventricles were obtained from litters corresponding to his $12^1/_3$- or $12^2/_3$-day stages. Chick ventricles were obtained from White Leghorn embryos ascertained to be stage 26 or 27 by the structure of their limbs.[13] Each group of ventricles was fragmented and incubated in 0.1% trypsin (Difco, 1:250) in calcium- and magnesium-free Hanks' solution at 37° for 25–60 min. After the fragments were rinsed in culture medium (Eagle's MEM with 10% horse serum and 100 units/ml of penicillin and 100 μg/ml of streptomycin), they were placed in fresh medium and dissociated into predominantly single cells by gentle vibration. Any large cell clumps present were removed by light centrifugation, and the cell concentrations were determined. Then the mouse and chick cells were mixed together in ratios ranging from 1:1 to 4:1 (mouse:chick) at total cell concentrations of 0.6–1.3 $\times$ 10^6 cells/ml. The cell suspensions were allowed to aggregate on a gyratory shaker[14, 15] at 37°. The aggregates were fixed in Bouin's fluid, and sections were prepared and stained with hematoxylin-eosin. Altogether, more than 275 aggregates were prepared in eight experiments.

In one experiment, the chick cells were labeled with tritiated thymidine. These labeled cells were obtained from embryos onto which 15 μc of thymidine-methyl-^{3}H (2c/mM, 150 μc/ml) were placed, through a window in the shell, at 75 and again at 98 hr of incubation.

Results.—Mouse and chick heart ventricles of the stages employed here appear comparable in their stage of development and cellular composition. In both, myocardial cells are the most numerous cell type; in addition, there are epicardial and endocardial cells and interstitial fibroblasts.

In the mouse-chick cell aggregates, mouse cells could be distinguished from chick cells by their larger and more deeply staining nuclei.[7, 16] In aggregates fixed eight hours after the cells were placed in the flasks, the cells of the two species were interspersed throughout each aggregate (Fig. 1*A*). However, in aggregates fixed after 2 to $2^1/_2$ days, extensive sorting-out of mouse and chick cells was observed (Fig. 1*B*). At the surface of these older aggregates, there was usually a one-cell-thick layer of flattened nonmyocardial cells. Internal to this thin capsule, the cells were almost exclusively myocardial. Within these myocardial areas, there was extensive segregation of the cells according to species. This sorting-out, while not quite as complete as the best cases of sorting-out that we and others have observed in aggregates of cells derived from two different chick tissues, was nevertheless obvious and unequivocal. Within the myocardial regions, often the mouse cells were at the periphery and the chick cells were located more internally. Because the layer of nonmyocardial cells was so thin, it was impossible to determine whether these cells also sorted out by species.

The nuclear size and staining differences between these mouse and chick cells seem to be fully adequate for distinguishing them. Nonetheless, to have an independent verification of cell origin, we studied mouse-chick ventricle aggregates in which the chick cells were labeled with tritiated thymidine. These aggregates were fixed after 49 hours in culture. Uncoated, conventionally stained sections were compared with adjacent sections coated with radioautographic emulsion and exposed for 14 days. We observed that the locations of patches of chick cells identified by their nuclear size and staining corresponded closely to the locations of patches of labeled cells in adjacent coated sections (Fig. 1*C* and *D*). Thus, the identification criteria do distinguish accurately between the cells of the two species.

Discussion.—Is it possible that the segregation of mouse and chick myocardial cells was due to extensive cell division, rather than to active sorting-out? If extensive cell division, but not active sorting-out, had been occurring here, the aggregates would have contained numerous small foci of mouse and chick cells. What was observed, however, was that almost all of the cells of each derivation were usually grouped in a few large clumps, often much too large to be explainable on the basis of cell division within the 2–$2^1/_2$-day period of culture.

Myocardial cells do not present the only case in which sorting-out between mouse and chick cells of the same kind has been observed. Okada[17] has found that mouse and chick embryonic kidney cells also sort out. How common, then, might the sorting-out capacity of corresponding mouse and chick embryonic cells be? We wish to suggest the possibility that the lack of sorting-out reported between mouse and chick limb precartilage and between mouse and chick liver may be due to special characteristics of these tissues rather than to identical "recognition" properties between the two species.

In the experiments with limb precartilage,[7, 8] the cells were taken from embry-

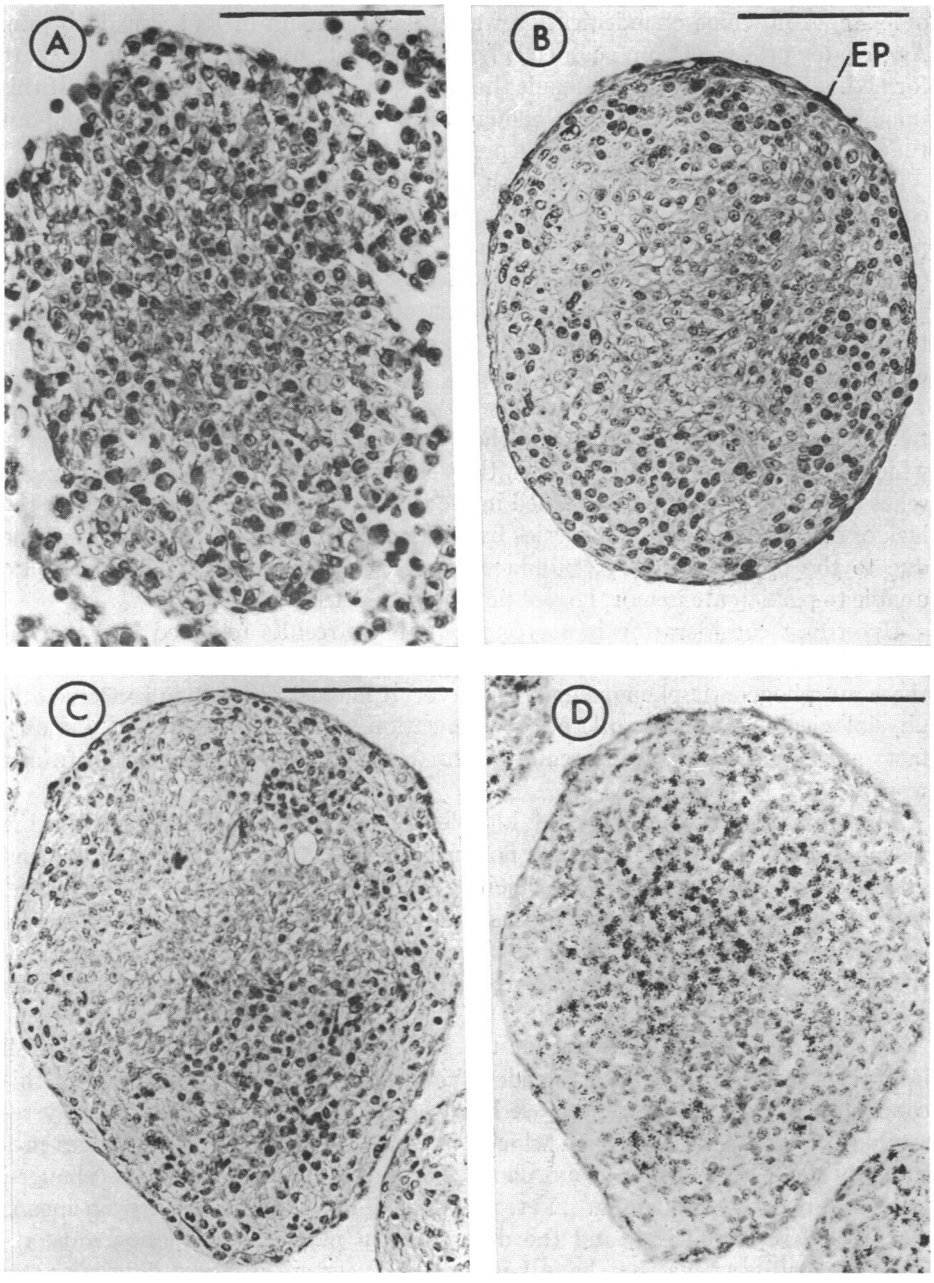

FIG. 1.—Mouse-chick heart ventricle cell aggregates. The mouse cell nuclei are larger and stain more deeply than the chick cell nuclei. The scale lines are 100 μ. (*A*) An aggregate fixed after 8 hr. (*B*) An aggregate fixed after 55 hr, with a one-cell-thick nonmyocardial epithelium (*EP*) at the surface surrounding the myocardial cell region. (*C*) A section of a 49-hr aggregate containing chick cells labeled with tritiated thymidine, but not coated with radioautographic emulsion. (*D*) A section next to the one shown in (*C*), coated with radioautographic emulsion.

onic stages in which chondrification was either imminent or had already begun. Aggregates prepared from such cells begin to secrete matrix soon after they are formed. It is likely that as a result the cells would become immobilized. If this immobilization took place early enough in mouse-chick aggregates, sorting-out that might otherwise occur would be prevented.

In evaluating the experiments with mouse and chick liver cells,[7, 8] one must consider the fact that mouse liver at the stages used is a very important hematopoietic organ, while this function is far less significant in chick embryonic liver.[18] Of the cells present in mouse embryonic liver, 35 to 65 per cent are not liver parenchymal cells at all, but rather are hematopoietic cells.[19] In contrast, hematopoietic cells represent at most only a small percentage of the cells found in chick liver at the stages used.[18]

In general, blood cells do not join with each other or with other types of cells to form coherent structures, nor is their locomotory behavior like that of cells which form coherent tissues. Thus, the very properties upon which sorting-out relies appear to be severely modified in the course of hematopoiesis. Hence, the lack of clear sorting-out on a species basis in mouse-chick liver aggregates may be due to the presence of large numbers of mouse hematopoietic cells which are unable to participate in morphogenetic processes of this kind.

Upon first consideration it may seem that the results reported here, by invalidating a widely held generalization, have decreased the order discernible in these morphogenetic phenomena. However, it is most difficult to conceive of a physiological basis for the original generalization, and all of the presently known facts are actually very well accounted for within a different conceptual framework.

The growing body of detailed knowledge concerning cell behavior during morphogenetic reorganizations can no longer be accommodated by a conceptual system whose categories are "specificities" organized according to tissue, stage, and species. These assembly processes are instead readily understood as responses of mobile cell populations to characteristic differences in the strengths of the different kinds of cell adhesions represented within a cell system (reviewed in ref. 6).

The differing results reported for the various mouse-chick combinations fall into place when evolutionary considerations are taken into account within this conceptual framework. The mouse heart and the chick heart still strongly resemble the reptilian heart from which both evolved. These resemblances presumably reflect the action of morphogenetic determinants which have changed but little over the millennia. Yet, the two kinds of hearts are by no means morphologically identical, and the differences in plan of construction must reflect the modifications that have been made in certain of these determinants. The same holds true for many other organs.

Modification of cellular adhesive properties has probably played an important role in bringing about morphogenetic evolution in some organs. In other organs, the most significant aspects of morphogenetic evolution could no doubt be traced to changes in morphogenetic determinants of a different nature. We therefore do not find it surprising that homologous embryonic mouse and chick organs in

some cases yield cells which sort out from each other readily, and in other cases evidently may not.

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